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1 A report on six cases of seagrass-associated gastric impaction in bottlenose dolphins (*Tursiops*
2 sp).

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21 Marine debris such as plastic and other foreign objects (*e.g.*, nylon fishing line, cigarette
22 wrappers, metal bottle caps) are commonly found in odontocete stomachs (Walker and Coe
23 1990), but due to the often small amounts and diversity of the debris, the impact on animal health

is unclear (Walker and Coe 1990, Laist 1997, Baird and Hooker 2000). Small quantities can however, have large effects if they are capable of intermittently or consistently blocking the pyloric outflow tract (*e.g.*, Tarpley and Marwitz 1993, Stamper *et al.* 2006, Jacobsen *et al.* 2010) causing long-term malnutrition and death (Gomerčić *et al.* 2006); either directly due to acute gastric rupture or complete pyloric obstruction, or indirectly as a result of chronic malnutrition due to partial/intermittent pyloric obstruction. Kastelein and Lavaleije (1992) documented the persistence of undigested algae in the forestomach of a harbor porpoise for at least 3 days. Several other odontocete species (*e.g.*, bottlenose dolphins (*Tursiops truncatus*, *e.g.*, McBride 1940), northern right whale dolphins (*Lissodelphus borealis*, Walker and Coe 1990), and spinner dolphins (*Stenella longirostris*, Trianni and Kessler 2002)) have been found to ingest marine plants, such as kelp (*Egregia* sp., *Macrocystis pyrifera*), seaweed (*Fucus vesiculosus*) or seagrass (*Enhalus acroides*, *Haldule uninervis*). However, death due to gastric impaction by marine plants (high densities of intertwined marine plant(s) preventing efficacious pyloric emptying and gastric evacuation (Santos *et al.* 2001)) is rarely reported (see McBride 1940, Trianni and Kessler 2002).

Here, we report the results of necropsies of bottlenose dolphins (*Tursiops* sp.) with gastric impaction due to seagrass ingestion. Descriptions of the stomach contents in cetaceans help us to determine diet and assess anthropogenic impacts (*e.g.*, if dolphins are ingesting debris, stealing bait, or changing prey due to depletion of favored prey species) and therefore direct conservation and management efforts. Although there are a number of biases associated with analysis of stomach contents for determination of diet (*e.g.*, differential prey digestion and retention, dead animals and stranding cases not being representative of whole populations), our central interest is in *relative* differences in seagrass content as a function of dolphin age. Specifically, we focus on mortality associated with ingestion of large amounts of seagrass, a rare

occurrence. We examined stomach contents of 40 dead bottlenose dolphins opportunistically found at two sites, Shark Bay and Bunbury, in Western Australia.

Shark Bay (SB: 25° S. 113° E) is a large (22,000 km²), semi-enclosed bay with two shallow embayments (< 16 m), 850 km north of Perth. Shark Bay has extensive shallow seagrass banks and is the site of long-term dolphin research, where the study population includes over 2,000 individually recognized bottlenose dolphins sighted since 1984. The main study site covers 300 km² in the eastern gulf and 250 km² in the western gulf. The Bunbury (BB: 33° S. 115° E) study site, 180 km south of Perth, covers 120 km² (maximum water depth is ~10 m) along 65 km of coast exposed to wave action with no barriers or islands for protection. The benthic habitat includes seagrass, limestone reef, macroalgae communities and sand (Hillman *et al.* 2000). The study population includes 259 individually recognized bottlenose dolphins identified between 2007 and 2010 (Smith, personal communication).

The taxonomy of SB and BB bottlenose dolphins is unresolved. The mitochondrial DNA haplotypes based on the control region of SB *Tursiops* are characteristic of both *T. aduncus* and *T. truncatus* (Krützen *et al.* 2004). Therefore, we refer to SB and BB dolphins as *Tursiops* sp., although in SB their speckling patterns are similar to those in *T. aduncus* (Ross and Cockcroft 1990, Krzyszczyk and Mann 2012); BB dolphins do not speckle with age (Smith, personal communication) and genetic investigations are underway.

The age of dead dolphins was estimated by known birth year or determined from thin sections of postnatal dentine observed in the teeth following Hohn *et al.* (1989). When birth year or a tooth was not available for aging, age was estimated by the degree of ventral speckling (Krzyszczyk and Mann 2012) and/or body size and length. Dolphins were placed in one of three categories: calf (0 to 4 yr), juvenile (sexually immature, 5 to 10 yr), and adult (mature, >10 yr)

(Mann *et al.* 2000, Krzyszczyk and Mann 2012). In our sample, 3 calves were approximately 4 yr old and could have been recently weaned (Mann *et al.* 2000). Sampling was opportunistic, with assistance from the Department of Environment and Conservation (DEC) in carcass collection and necropsies. Standard morphometric measurements were recorded from stranded carcasses during necropsies with supporting information including location, sex, degree of ventral speckling, external wounds and scars, and date of stranding for each dolphin.

Of the 40 dead bottlenose dolphins (*Tursiops* sp.) found, 30 were in SB (1992-2010), and 10 in BB (2007-2010). Over half (52.5%) were calves or juveniles, 30% were adults, and 17.5% were of unknown age. Of the 40 dead dolphins, 27 complete or partial necropsies were performed by veterinarians and researchers (see Table S1 for details). Eight stomachs contained seagrass (Table 1); one adult (ID: 99-10) and 7 calves (ID's: 09-665 to 03-15). Six of the 8 stomachs were impacted with seagrass (Fig. 1). All individuals with seagrass-associated gastric impaction were emaciated. The data suggest that all dolphins with gastric impaction were quite young, but with a small sample size, the pattern is not statistically significant (Fisher Exact Test, $P=0.136$).

Various reasons for cetacean debris ingestion have been proposed. For example dolphins might confuse debris with prey (*e.g.*, Derraik 2002), but given delphinid echolocation capabilities, this seems unlikely (Walker and Coe 1990). Here we focus on why dolphins might ingest large quantities of seagrass.

Dolphins commonly hunt in seagrass beds (Grigg and Markowitz 1997, Barros and Wells 1998, Mann and Sargeant 2003), and immature dolphins play with detached seagrass floating at the surface (Mann and Smuts 1999). Thus seagrass could be incidentally ingested when hunting or playing with seagrass (McBride 1940). For the adult (ID: 99-10) and calf (ID: 03-15) that had

only small amounts of seagrass in their stomachs, incidental ingestion during hunting or playing seems likely. However, mistaken or accidental ingestion does not explain the vast amount of seagrass in the engorged and impacted stomachs of the six affected calves (ID's: 09-665 to 92-1).

Walker and Coe (1990) suggested that debris ingestion in stranded cetaceans may be part of the stranding syndrome, and that naturally occurring disease factors may predispose an individual to ingest abnormal objects. Similarly, Kastelein and Lavaleije (1992) suggested that an individual weakened by lung parasites (or arguably any incapacitating chronic disease), might be rendered incapable of catching enough fish, and as a substitute, may start to eat whatever material it encounters. This may result in a false sensation of satiation for the animal, which would subsequently reduce the dolphin's appetite and meal size. Long-term, this could compromise the ability to form/maintain adequate blubber adipose stores; with chronic malnutrition potentially adversely affecting the health of the animal and subsequently (albeit indirectly), leading to its death. However, given that all of the dolphins with seagrass-associated gastric impaction were young, separation from the mother seems another likely cause of seagrass ingestion, leading to emaciation secondary to chronic malnutrition and eventually, death.

The sheer quantity of seagrass present combined with the fact that there were no obvious signs of milk in the stomachs of most of the calves affected by seagrass-associated gastric impaction suggests that their mothers were absent for hours to days prior to their death. Learning to hunt is a slow process, with calves successfully catching fish in their 4th month of life, but continuing to nurse for up to 8 years (Mann and Sargeant 2003; Mann *et al.* 2000). However, dolphins are precocious, often separating from their mothers for brief periods soon after birth (Mann and Smuts 1999) and continuing to do so until weaned (Gibson and Mann 2008, Stanton *et al.* 2011). Temporary separations entail risk of permanent separation. SB calves occasionally

appear to lose track of their mothers during long distance separations and it can take 2-3 hours before becoming reunited (Mann, personal communication).

We therefore suggest that maternal loss by either separation or death was the main factor causing calves to ingest seagrass, perhaps to feel sated because their hunting skills were not adequately developed. The result underscores the importance of the calf period for developing hunting skills (Sargeant and Mann 2009, Mann *et al.* 2007) and helps explain why bottlenose dolphin calves have some of the longest periods of dependence documented to date (Mann *et al.* 2000).

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Figures

Figure 1. Seagrass associated impacted forestomach of dolphin calf 06-18.

Tables

Table S1. Information on dolphins reported in this study. Location, age, age class, sex data and whether stomachs contained seagrass for 27 individuals on which necropsies were performed (BB = Bunbury, SB = Shark Bay).

228 *Table 1.* Summary of the 8 individuals with seagrass found in their stomachs (BB = Bunbury, SB = Shark Bay).

ID	Location	Sex	Age	Weaned	Stomach content	Stomach condition	Body condition/ other illness	Potential cause of death
09-665	BB	M	4 yr	Yes	Seagrass (<i>Posidonia</i> sp.), fine clay-like mud, fish otoliths	Phytebezoar with large amounts of clay-like mud in forestomach (weight = 4.9kg; dimensions = 36 x 23 x 12cm)	Extremely emaciated, external epidermal lesions, internal parasitism, contained Nematode sp. and Trematode sp.	Respiratory failure due to nematode-associated bronchopneumonia, poor body condition
09-1	SB	F	3-4 wks	No	Full of Seagrass (<i>Posidonia australis</i>)	Phytebezoar (dimensions = 12 x 9cm)	Emaciated	Maternal loss
06-17	SB	F	4-6 mo	No	Full of Seagrass (<i>Amphibolis antarctica</i>)	Phytebezoar, inflamed pyloric stomach	Extremely emaciated	Maternal loss
06-18	SB	F	4-6 mo	No	Full of Seagrass (<i>Posidonia</i> sp.)	Phytebezoar in forestomach	Emaciated	Maternal loss
08-21	SB	M	6 mo	No	Full of seagrass (<i>Cymodocea argustata</i> and 3 other sp.)	Phytebezoar in forestomach	Emaciated	Maternal loss
92-1	SB	F	~1.5 yr	No	Full of Seagrass	Phytebezoar	Extremely emaciated	Maternal loss

03-15	SB	M	2 yr	No	Residue of milk, small bundle of seagrass	No overt gastric impaction or pathology	Hepatic fracture and hemorrhage with overlying subcutaneous and intramuscular hemorrhage	Blunt trauma
99-10	SB	M	~30 yr	Yes	Some strands of seagrass, lots of small fish and bones	No overt gastric impaction or pathology	Extremely emaciated	Respiratory failure due to nematode- associated pneumonia, poor body condition